

## **A Metal Binding Site in the Core of a Tetrameric Coiled-Coil appears to be Important for Determining the Oligomeric State**

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Beamline(s): **X12C**

**Introduction:** NSP4 is a rotavirally-encoded protein, which is both essential for viral maturation and appears to be the causative agent responsible for enterotoxic symptoms associated with rotavirus infections. We have determined the crystal structure of the oligomerization domain of this protein, which was previously predicted to form a tetrameric coiled-coil.

**Methods and Materials:** Crystals of a synthesized peptide spanning residues 95-137 (NSP4[95-143]) with a single selenomethionine at position 112 were grown in 22-30% PEG400 and a variety of mono and divalent salts. Multiple datasets were collected on X12C using two crystals grown in magnesium sulfate and strontium chloride, respectively.

**Results:** Experimental MAD-phased electron density maps indicate the presence of a divalent cation bound in the core of the tetrameric coiled-coil coordinated by four glutamine and two glutamate residues. Despite the high concentration of magnesium (320mM) in the first crystal, the organization of the metal-binding site strongly suggests the presence of a calcium ion, which is supported by the increased density of the strontium ion in the second crystal. The orientation of the helices at and C-terminal to the metal binding site is as expected for a tetrameric coiled-coil; however, the region N-terminal to the metal-binding site displays significant twisting of the helices with respect to each other.

**Conclusions:** The presence of a divalent cation neutralizes the charge of two of the four core glutamate residues, and in doing so allows these residues to be pointed inwardly and offers a bridging density across the core. There is a significant distortion of the helical arrangement amino-terminal to this metal-binding site. Given this nonidealized arrangement, coordination of a divalent cation may be required for the tetrameric organization observed here.

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